(ii) a nucleic acid comprising a nucleotide sequence corresponding to the nucleic acid of (i) within the scope of the degeneracy of the genetic code, and

(iii) a nucleic acid comprising a nucleotide sequence which hybridizes with at least one of the nucleic acids of (i) or (ii) under stringent conditions;

(b) culturing the host cell under conditions which induce expression of the nucleic acid and production of the corresponding protein, and

(c) isolating the protein from the host cell.--

REMARKS

The Office Action dated July 16, 2001 has been received and carefully noted. The above amendments and the following remarks are submitted as a full and complete response thereto.

Claims 1-17, 19-24, 26, 28-30, 32-34 and 37-65 are all the pending claims in this application. By this Amendment, claims 1 and 5-12 have been amended, support for which can be found as follows:

Claim 1 is now directed to a "complete S-layer protein" (see, for example, page 17, line 28 continued to page 18, line 2 and/or page 18, lines 6-10 of the specification); and

Claims 5-12, which depend from claim 4, have been amended to recite the phrase "at least one insertion" which brings the claims into proper dependent format.

New claim 66 finds support, *inter alia*, in the subject matter of claims 1 and 4 as submitted in the Amendment of April 23, 2001.

All of the claims find support in the specification as filed and no new matter has been added. Accordingly, consideration and entry of the amended claims and new claim 66 is requested.

I. Rejection of Claims 5-12 under 35 U.S.C. §112, second paragraph

Claims 5-12 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite.

Claims 5-12, which depend from claim 4, recite the limitation "insertions", and the term lacks antecedent basis for this limitation in claim 4; claim 4 recites "insertion".

Applicants have amended claims 5-12 to recite “at least one insertion”.

II. Withdrawal of Rejections

Applicants gratefully acknowledge the Examiner's withdrawal of the objection and rejection under 35 U.S.C. § 112, first paragraph.

III. Response to Rejection of Claims 1, 3, 15-17, 19, 20, 46, 47 and 58-63 under 35 U.S.C. §102(b)

Claims 1, 3, 15-17, 19, 20, 46 and 47, and now 58-63 are rejected under 35 U.S.C. §02(b) as being anticipated by Kuen et al (Gene, 1994).

The Examiner alleges that in view of Kuen disclosing

a) cloning and expression of a 5-layer protein in a prokaryotic cell system;

b) the nucleic acid sequence of the 5-layer protein including the signal sequence of the sbs gene (abstract; p. 116, col. 1);

c) cells for transformation and

d) a vector (p. 116, Experimental and Discussion),

Kuen anticipates the claimed invention.

Applicants traverse the rejection of the claims for the following reasons.

Applicants respectfully submit that the Examiner's interpretation of Kuen is technically incorrect. The Examiner concludes from Kuen's efforts in subcloning the sbsA gene in fragments and sequencing those parts and assembling from those partial sequences, that Kuen actually discloses having cloned the complete, full-length or contiguous nucleotide sequence for the sbsA gene. Kuen may disclose that the S-layer protein was cloned and expressed in a prokaryotic cell system, but Kuen did not clone or express a full length gene as originally obtained from a full length clone of the gene.

Thus, it cannot be concluded from the teachings of Kuen that the entire or full-length S-layer gene was cloned much less that it was expressed in *E. coli* or any other prokaryotic cell for that matter. The Summary of the Kuen publication states explicitly:

> " ... the entire nucleotide (nt) sequence of the sbsA was determined from three overlapping fragments."

This means that partial sequences were assembled to give a complete but nevertheless theoretical sequence. Kuen goes on to state:

> "The 3'-end was cloned and expressed in Escherichia coli, whereas the 5'-region was amplified from the genome of Bs PV72 by the polymerase chain reaction using two overlapping fragments."

Thus, only an incomplete fragment of the S-layer protein was ever produced in a prokaryotic system.

Cloning of the two DNA sequences which were produced via PCR and which encode various parts of the 5'-region of the sbsA gene, was effected in a pUC18 vector for the purpose of sequencing. Expression in pUC18 would not have been technically

possible because the subcloned gene would not have been "in frame" and would have resulted in expression of an "out of frame" S-layer protein. SbsA expression was never accomplished by Kuen, and therefore, Kuen cannot even be considered as having disclosed the original full length gene much less a recombinant form of the protein encoded thereby.

Thus, Kuen did not successfully clone the full length sbsA gene but rather numerous subcloned fragments using various cloning vectors. By contiguous analysis, the subcloned fragments were then used to generate a complete sequence. Expression of a full-length S-layer protein in *E. coli* is not described by Kuen et al. As previously explained, only the C-terminal part of the S-layer protein was cloned and expressed by Kuen in a prokaryotic cell system.

Additionally, Kuen does not disclose an expression vector suitable for producing an S-layer protein. On the contrary, Kuen specifically discourages expression in an *E. coli* host cell stating that expression of a complete S-layer gene in E. coli should be impossible due to toxicity or instability.

Additionally, Applicants submit that only after cloning the full length sbsA gene and after expression of the full gene in *E. coli*, was the surprising discovery made that the S-layer protein actually forms the same S-layers in the cytoplasm of a gram-negative cell system as found on the cell surface of gram-positive organisms.

In view of the foregoing comments and discussion, Applicants submit that the claimed invention is novel, and that withdrawal of the anticipation rejection is deemed proper.

IV. Respons to R j cti n of Claims 1-17, 19, 20, 46, 47 and 58-65 under 35 U.S.C. §103(a)

Claims 1-17, 19, 20, 46 and 47, and now 58-65 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kuen et al (Gene, 1994) taken with Deblaere et al (W09519371).

In the Office Action, the Examiner alleges that the instant claims are *prima facia* obvious over the methods/processes taught by both Kuen and Deblaere since one skilled in the art would have had a reasonable expectation of success in obtaining a process to produce pure 5-layer protein, a recombinant 5-layer protein or a fusion protein comprising the 5-layer protein having an insertion of a heterologous polypeptide as presently claimed.

Applicants traverse for the reasons set forth under section III with respect to Kuen, and for the reasons set forth below in view of the combined reference rejection.

Only parts of the gene were cloned by Kuen, which makes it more than apparent that Kuen could not possibly have described an expression vector for the S-layer gene. Therefore, combining the disclosure of Kuen with Deblaere is inadmissible, if only for this reason.

Additionally, the Examiner's allegation that Deblaere discloses expression of a recombinant S-layer protein with inserted foreign sequences in gram-positive and gram-negative bacteria is technically incorrect. Page 12 of the patent states:

> "This host cell is generally a bacterium, typically a bacterium which naturally produces a S-layer protein, i.e. a bacterium which in its native state has a S-layer **on its surface**. Depending upon the intended use,

the bacterium may be a gram-positive or gram-negative bacterium."

Deblaere only discloses expression and presentation of a recombinant Caulobacter S-layer protein **on the surface** of gram-positive bacteria and *E. coli* is specifically excluded. Therefore, it is not even conceivable that Deblaere could render obvious the instant claimed invention for a functional S-layer protein having functional recombinant insertions and formed in the cytoplasm of an *E. coli*.

Moreover, Deblaere only describes N-or C-terminal fusions of the S-layer protein with heterologous fusion partners. In contrast, the present specification describes recombinant sbsA S-layer proteins having one or more heterologous insertions. The skilled artisan would fully appreciate the technical distinction between an "insertion" and a terminal fusion as disclosed by Deblaere.

For all of the above cited reasons, the instant claimed invention is nonobvious over Kuen alone or in combination with Deblaere, and withdrawal of the rejection is deemed proper.

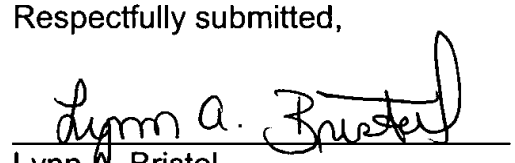
CONCLUSION

In view of the amended claims and the foregoing arguments, Applicants submit that the Examiner's rejections are met and overcome. The claims are now in condition for allowance, and Applicants request that the Examiner allow the application to pass to issuance.

If for any reason the Examiner determines that the application is not now in condition for allowance, it is respectfully requested that the Examiner contact, by telephone, Applicant's undersigned attorney at the indicated telephone number to arrange for an interview to expedite the disposition of this application.

In the event this paper is not being timely filed, the applicant respectfully petitions for an appropriate extension of time. Any fees for such an extension together with any additional fees may be charged to Counsel's Deposit Account 1-2300.

Respectfully submitted,



Lynn A. Bristol
Registration No. 48,898

ARENT FOX KINTNER PLOTKIN & KAHN, PLLC
1050 Connecticut Avenue, N.W., Suite 400
Washington, D.C. 20036-5339
Tel: (202) 857-6000
Fax: (202) 638-4810

LAB/ccd

Enclosure: marked up copy of claims

1. (Twice Amended) A process for production of [an] a complete S-layer protein comprising

(a) transforming a gram-negative prokaryotic host cell with a nucleic acid encoding an S-layer protein selected from the group consisting of

(i) a nucleic acid comprising a nucleotide sequence from position 1 to 3684 of SEQ ID NO.1,

(ii) a nucleic acid comprising a nucleotide sequence corresponding to the nucleic acid of (i) within the scope of the degeneracy of the genetic code, and

(iii) a nucleic acid comprising a nucleotide sequence which hybridizes with at least one of the nucleic acids of (i) or (ii) under stringent conditions;

(b) culturing the host cell under conditions which induce expression of the nucleic acid and production of the corresponding protein, and

(c) isolating the protein from the host cell.

5. (Twice Amended) The process as claimed in claim 4, wherein the [insertions] at least one insertion are selected from the group consisting of nucleotide sequences encoding cysteine residues, regions with several charged amino acids or tyrosine residues, DNA-binding epitopes, metal-binding epitopes, immunogenic epitopes, allergenic epitopes, antigenic epitopes, streptavidin, enzymes, cytokines, and antibody-binding proteins.

6. (Twice Amended) The process as claimed in claim 5, wherein the [insertions] at least one insertion encode streptavidin.

7. (Twice Amended) The process as claimed in claim 5, wherein the [insertions] at least one insertion encode immunogenic epitopes from a herpes virus.

8. (Twice Amended) The process as claimed in claim 5, wherein the [insertions] at least one insertion encode enzymes comprising polyhydroxybutyric acid synthase or bacterial luciferase.

9. (Twice Amended) The process as claimed in claim 5, wherein the [insertions] at least one insertion encode cytokines comprising interleukins, interferons or tumour necrosis factors.

10. (Twice Amended) The process as claimed in claim 5, wherein the [insertions] at least one insertion encode antibody-binding proteins comprising protein A or protein G.

11. (Twice Amended) The process as claimed in claim 5, wherein the [insertions] at least one insertion encode antigenic epitopes which bind cytokines or endotoxins.

12. (Twice Amended) The process as claimed in claim 5, wherein the [insertions] at least one insertion encode metal-binding epitopes.